Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Different frequency of epidermal growth factor rs76189946 polymorphism genotype in Iranian colorectal cancer patients

Vahid Chaleshi[1], Sanaz Savabkar[1], Hesamodin Dolatmoradi[1], Mahdi Montazer Haghighi[2], Elmira Khalili Maryan[1], Sara Pasha[3], Narges Ebrahimi Vandfaez[1], Ehsan Nazemalhosseini Mojarad[3], Hamid Asadzadeh Aghdaei[1], Mohammad Reza Zali[3]

[1]Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2]Department of Biology, Science Faculty, Islamic Azad University, East Tehran Branch, Tehran, Iran

[3]Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Aim: This study aimed to determine the association between rs76189946 polymorphism of EGF gene and the risk of colorectal cancer in an Iranian population.

Background: Colorectal cancer (CRC) is the third most prevalent cancer in both genders worldwide. The determination of genetic variation becomes a new way to the etiology of colorectal cancer. Epidermal growth factor (EGF) is a mitogen that plays an important role in cell growth and tumourigenesis. This protein acts by binding its receptor, EGFR.

Patients and methods: DNA samples taken from totally 125 CRC patients and healthy controls were amplified by polymerase chain reaction (PCR) for the rs76189946 polymorphism. Genotypes were analyzed using restriction fragment length polymorphism (RFLP). Finally to confirm the RFLP procedure, 20 of the PCR products were sequenced using the ABI PRISM 3130xl genetic analyzer and chain termination method (Applied Biosystems, Carlsbad, CA).

Results: Genotype distribution and allele frequency was similar in CRC patients and controls individuals. We expect to observe C and G allele in both groups, but only found C allele.

Conclusion: In this study for the first time we identified genetic distribution of exonic rs76189946 polymorphism in EGF gene both CRC patients and healthy controls. These results suggest there was no association between EGF polymorphism rs76189946 and risk of colorectal cancer in an Iranian population.

Keywords: Colorectal cancer, Single nucleotide polymorphism (SNP), rs76189946, Epidermal growth factor (EGF).

(Please cite as: **Chaleshi V, Savabkar S, Dolatmoradi H, Montazer Haghighi M, Khalili Maryan E, Pasha S, et al. Different frequency of epidermal growth factor rs76189946 polymorphism genotype in Iranian colorectal cancer patients. Gastroenterol Hepatol Bed Bench 2013;6(Suppl 1):S32-S38).**

Introduction

Colorectal cancer (CRC) is one of the most common cancer in the world and the third leading cause of cancer deaths (1). Some studies are shown that CRC developing in Asian countries (2-4). The etiology of CRC is very complicated and associated with different genetic features (5). Epidermal growth factor (EGF) is a one of the mitogens that belong to EGF super family (6). The human tumor cells release growth factor that may play a role in the pathogenesis of cancer (7). The *EGF* gene is located on chromosome 4q25-27 that

Received: 28 April 2013 *Accepted*: 8 July 2013

Reprint or Correspondence: Hamid Asadzadeh Aghdaei, MD. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

E-mail: hamid.assadzadeh@gmail.com

alternative splicing produces different transcripts. However, the product of EGF gene is twelve transcripts, which include three coding transcripts, and other transcripts have no protein products (http://asia.ensembl.org/Homo_sapiens/Gene/Summary) (figure 1). Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor and broadly expressed on several cell types, including epithelial and mesenchymal celsl (8). Interaction of EGF/EGFR lead to dimerization of EGFR and activated several signaling pathway (PI3K/AKT, RAS/ERK and JAK/STAT) that cause proliferation, differentiation and stimulate mitogenesis in epithelial cells (5, 9, 10). Tumor cells synthesize high levels of growth factors regulating for proliferation and survival (11, 12). Single nucleotide polymorphisms (SNPs) are the most common cause of human genetic alternation and may contribute in susceptibility and intensity of the disease (13). The relation between *EGF* rs4444903 polymorphism located in 5′UTR and several cancers were investigated including, colorectal cancer (14), prostate cancer (15), melanoma (16), ovarian cancer (17) and breast cancer (18). According to our knowledge several single nucleotide polymorphisms have been identified in *EGF* gene region but a few studies have reported of *EGF* gene exonic polymorphisms and the coloration between the types of cancer (19, 20). In this study we investigated coloration between rs76189946 polymorphism that located in exon 23 of *EGF* gene and risk of colorectal cancer for the first time in an Iranian population.

Patients and Methods

Study population

This case-control study was carried out from 2006 to 2011 with patients attending the colorectal cancer unit of the Taleghani University Hospital

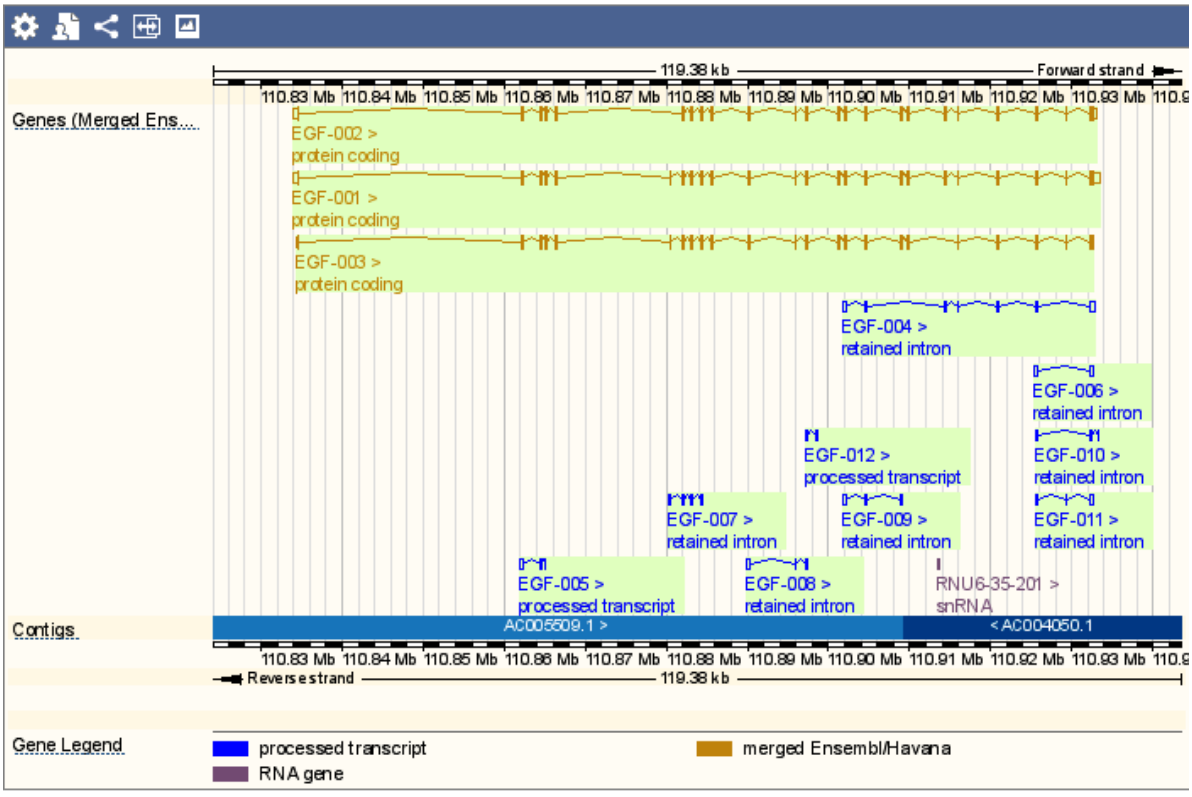

Figure 1. Region detail of *EGF* gene
(http://asia.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG00000138798;r=4:110834040-110933422)

and included healthy individuals to assess the risk association of colorectal cancer and *EGF* rs76189946 polymorphism. After appropriate informed consent, a total of 125 subjects (including 30 colorectal cancer patients and 95 healthy individuals) were studied. This study was conducted under the approval of the ethics committee of the Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences (Tehran, Iran).

DNA extraction

A 5ml venous blood was collected in EDTA-containing tubes and genomic DNA extracted from mononuclear cells by salting out method (21). The quality of the extracted DNA was then assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, inc., Wilmington, DE, USA).

rs76189966 polymorphism genotyping

Obtained DNA was used to determine the *EGF* rs76189966 polymorphism using the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP). To amplify DNA segment, the specific primers were designed (Table 1).

Table 1. Primer sequence and resulting fragment length for growth factor gene polymerase chain reaction (PCR)

	Primer direction	Primer sequence	GC %	Resulting fragment bp
1	Forward	5'- GTTTAGGGTCTGTCTTGC -3'	50	434
2	Reverse	5'- TCGTAGAGATTGTGGAACC -3'	47.3	

Amplification, PCR was carried out in a 25 µL mix containing 100 ng genomic DNA, 1xTaq Buffer, 0.5 mM of each deoxyribonucleotide triphosphate (dNTP), 1 mM of each primer, and 1U Taq DNA Polymerase. The PCR cycling conditions were 5 min at 94°C, followed by 32 cycles of 45 s at 94°C, 40 s at 57°C and 45 s at 72°C, with a final elongation step at 72°C for 5 min. For RFLP, the PCR products were digested with HaeIII (New England Biolabs, 5 U at 37°C for 6 h). The digested PCR products were run on a 3.5% agarose gel and stained with green viewer for visualization under UV light.

Sequencing

To confirm the RFLP procedure, 16% of the PCR products were sequenced using an ABI PRISM 3130xL Genetic analyzer (Applied Biosystems®, Invitrogen Life Technologies, Carlsbad, Ca, USA) and the chain termination method.

Results

Between 2009 and 2011 we totally recruited 125 individuals (including 30 sporadic colorectal cancer patients and 95 healthy controls). At the time of diagnoses, mean age of patients was 61.21±13.5 and in control individuals the mean age was 43.86±1.82 years. The gender distribution for the males comprised 66.67% of cases and 43.2% of control and female distribution including 33.33% of cases and 56.8% of control. After enzyme digestion, the genotype CC in 287+147 bp lengths, genotype CG in 287+245+147+47bp lengths and for genotype GG 245+147+47bp lengths were observed. *EGF* genotype was successfully determined in all patients and controls. In the entire patient and control group population, the C/G and G/G genotypes were not observed. Also, in both groups we found only C allele. After a randomly direct sequence of 20 PCR products, all selected sample showed that only CC genotype, which this result confirmed the RFLP procedure (Figure 2 and 3).

Table 2. Characteristics of studies according to single nucleotide polymorphism (SNP) in different region of *EGF* gene.

Author	SNP	region	Correlation	Publication year	population
Shahbazi et al (16)	rs4444903	5´UTR	malignant melanoma	2002	European
Zhen Zhan et al (19)	rs2237051	Exon	gastric cancer	2012	Chinese
	rs3733625	3´UTR	gastric cancer	2012	Chinese
Chaleshi et al (29)	rs2298979	Intron	colorectal cancer	2013	Iranian
Jia Wu et al (20)	rs11569017	Exon	Hepatocellular Carcinoma	2013	Chinese

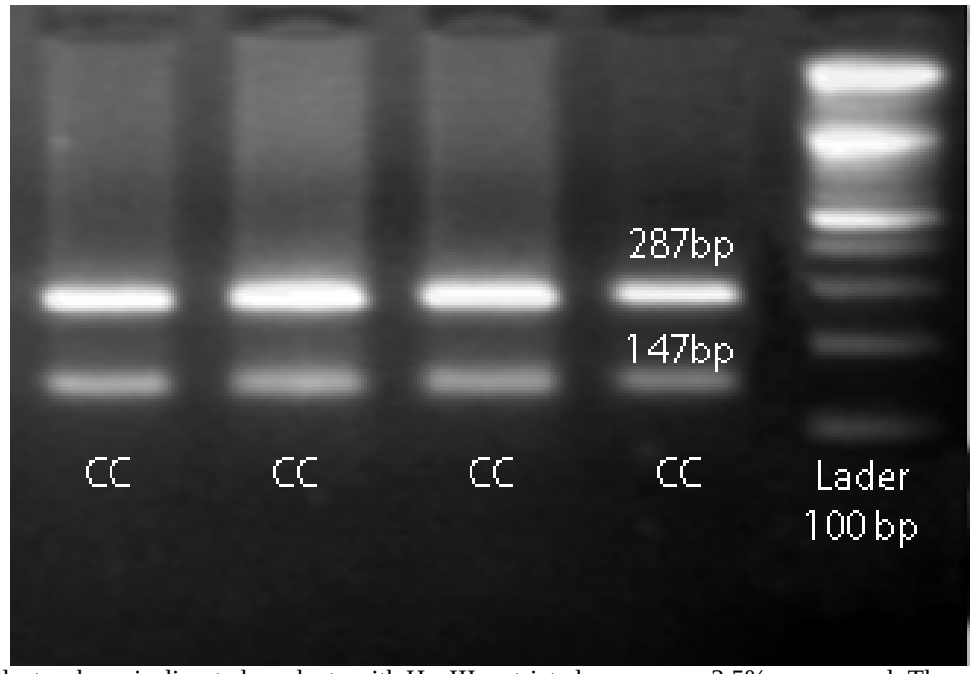

Figure 2. Electrophoresis digested products with HaeIII restricted enzyme on 3.5% agarose gel. The marker (M) that used was 100 base pairs, all sample showed CC genotype.

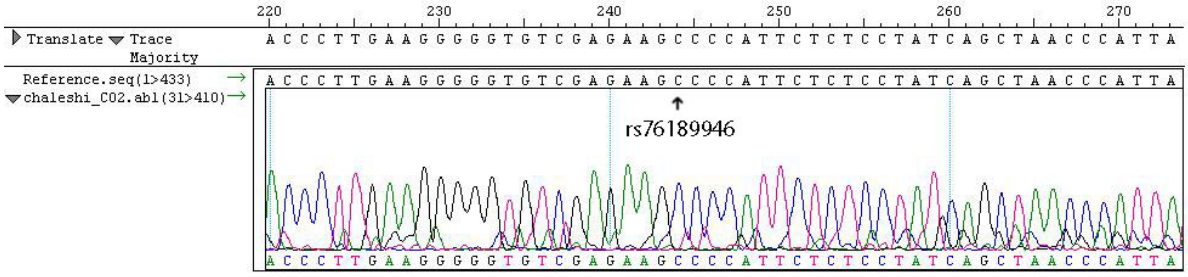

Figure 3. Direct DNA sequencing results for the epidermal growth factor (EGF) rs76189946 C/C genotypes.

Discussion

The EGF provide a prominent signaling pathway in colorectal cancer, as it increased proliferation, differentiation and survival in tumor cells (10). According to a previous study EGFR expression elevated in 60-80% of colorectal tumors (22). Hence, performed to investigate the EGF genetic association study appears obligatory. SNPs are the widespread kind of genetic variation they can function as biological marker and detecting cancer gene(23). Influence of single nucleotide polymorphism variation regarding the

increase or decrease of the risk of colorectal cancer development has been demonstrated by several studies in Iranian population (5, 24, 25). SNPs can absorb in promoter region of the gene that may be change the gene expression (26). Further Studies focus on association between rs4444903 SNP in the promoter region of *EGF* gene and types of cancer. In a meta-analysis study by Ying Piao et al suggested that G allele and GG genotype of rs4444903 polymorphism has correlations with esophageal and colorectal cancer(27). Furthermore, another study on the *EGF* rs4444903 polymorphism by Spindler, colleagues support the hypothesis that *EGF* rs4444903 polymorphism in the promoter region could change gene expression that these findings can be concerning the role of EGF factors (28). Although in our previous study, we did not find an association between the rs4444903 variants and the risk of colorectal cancer (14). A few studies showed that the SNPs located in other regions of *EGF* gene correlated with some diseases (table 2) (19, 20, 29). The study by Zhen Zhan et al indicate that a change in amino acids from isoleucine to methionine of *EGF* rs2237051 polymorphism correlated with an increased risk of intestinal gastric cancer and rs3733625 in the 3'UTR of *EGF* gene may contribute to the etiology of intestinal gastric cancer in the Chinese population(19). In our previous study for the first time shown the rs2298979 located on the intron region of *EGF* gene is correlated with colorectal cancer. Although we find no individuals with a G/G genotype despite the fact that the frequency of the G and A alleles was similar in the healthy control and CRC patient groups (14). In this case-control study for the first time we investigated association between rs76189946 and the risk of colorectal cancer in an Iranian population. *EGF* gene polymorphism rs76189946 is a missense mutation in amino acid position 1141, which changes Pro to Ala amino acid of the protein. According to NCBI database submitted dbSNP_ind_id: 30346 in European population the Average Het.+/- std err for rs76189946 is 0.500+/-0. However, our findings show different SNP frequency of genotypes. In this present study, we expected see two allele variations (C, G) of rs76189946 in the selected population but our results showed only CC genotype and C allele in all subjects. Some events such as ethnic heterogeneity, genotype distributions, gene environment interactions, different sample size and natural selection defined as a cause of this difference in world population (14, 30). Recently, a study has been suggested that negative selection reduced population differentiation in gene regions and in contrast positive selection leads to increasing population differentiation in gene regions mainly variation at nonsynomous and 5'-UTR (30).

In conclusion, our study shows that the exonic rs76189946 polymorphism in *EGF* gene has no significant association with CRC patients. More samples in further investigation studies with ethnically diverse populations are required to confirm our findings.

Acknowledgements

We would like to thank all patients who participated in the study, which was conducted with the support of Shahid Beheshti University of Medical Sciences.

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